Amendments to the Specification

Please replace the paragraph beginning at page 2, line 22 and ending at page 4, line 8 as follows:

Phosphopeptides can be synthesized in general by two strategies: a building block approach and a global phosphorylation method (Perich, J. W. (199l)[[.]] Synthesis of ~~0~~O-Phospho serine-Containing and ~~0~~O-Phosphothreonine-Containing Peptides[[.]], Methods in Enzymology 201, 225-233; Perich, J. W. (1991)[[.]] Synthesis of ~~0~~O-Phosphotyrosine-Containing Peptides[[.]], Methods in Enzymology 201, 234-245; Perich, J. W. (1997)[[.]] Synthesis of phosphopeptides using modem chemical approaches[[.]], In Solid-Phase Peptide Synthesis, Methods in Enzymology 289, pp. 245-266). The building block approach involves the stepwise incorporation of protected phosphoamino acids at the peptide synthesis stage. Incorporation of Fmoc-phospho tyrosine without phosphate ester protection (Ottinger, E, A. (1996)[[.]] Peptide Research 9, 223) in Fmoc based peptide synthesis has been reported. In addition, the use of the methyl (Valerio, R. M., Perich, J. W., Kitas, E. A., Alewood, P. F., and Johns, R. B. (1989)[[.]] Synthesis of ~~0~~O-Phosphotyrosine-Containing Peptides [[.]]2[[.]], Solution-Phase Synthesis of Asn-Glu- Ptyr-Thr-Ala Through Methyl Phosphate Protection[[.]], Australian Journal of Chemistry 42, 1519- 1525), benzyl (Kitas, E. A., Knorr, R., Trzeciak, A., and Bannwarth, W. (1991)[[.]] Alternative Strategies ~~For~~ for the Fmoc Solid-Phase Synthesis of O-4-Phospho-L-Tyrosine-Containing Peptides[[.]], Helvetica Chimica Acta 74, 1314-1328; Kitas, E. A., Wade, J. D., Johns, R. B., Perich, J. W., and Tregear, G. W. (1991)[[.]] Preparation and Use of N-Alpha-Fluorenylmethoxycarbonyl-~~0~~ O- Dibenzylphosphono-L-Tyrosine in Continuous-Flow Solid-Phase Peptide-Synthesis[[.]], Journal of the Chemical Society-Chemical Communications, 338-339), allyl and t-butyl (Perich, J. W., and Reynolds, E. C. (1991)[[.]] Fmoc Solid-Phase Synthesis of Tyr(P)-Containing Peptides Through Tert-Butyl Phosphate Protection[[.]], International Journal of Peptide and Protein Research 37, 572- 575) phosphate esters in Fmoc synthesis have been reported. Peptide synthesis with FmocTyr(PO_3H_2) has been reported for the introduction of phosphotyrosine (Ottinger, E. A., Shekels, L. L.,

Bernlohr, D. A., and Barany, G. (1993)[[.]] Synthesis of Phosphotyrosine-Containing Peptides and Their Use As Substrates For Protein-Tyrosine Phosphatases[[.]], Biochemistry 32, 4354-4361). This amino acid derivative appears to be suitable for the synthesis of small (less than 10 residues) phosphopeptides in good yield by standard Fmoc-SPPS techniques. However, couplings to form amide bonds using this derivative tend to be sluggish. Peptides with adjacent phosphotyrosine residues also tend to undergo condensation, forming a pyrophosphate derivative (Garcia Echeverria, C. (1995)[[.]] Letters in Peptide Science 2,93; Ottinger, E. A. (1996)[[.]] Peptide Research 9,223). In recent years, the use of the mono-phosphate protected species FmocTyr[PO(OBzl)OH] has gained prominence (White, P., and Beythien, J. (1996)[[..]] In Innovations ~~8c~~ and Perspectives in Solid Phase Synthesis & Combinatorial Libraries, 4th International Symposium, R. Epton, ed. (Birmingham: Mayflower Scientific Ltd.), pp. 557). This tyrosine derivative exhibits improved reactivity and solubility properties relative to FmocTyr(PO_3H_2). Phosphoserine derivatives can undergo p-elimination to the corresponding dehydroalanine species on treatment with piperidine in the standard Fmoc removal protocol. This elimination is rapid if the phosphate is bis-protected (Lacombe, J. M., Andriamanampisoa, F., and Pavia, A. A. (1990)[[.]] Solid-Phase Synthesis of Peptides Containing Phosphoserine Using Phosphate tert-Butyl Protecting Group[[.]], International Journal of Peptide and Protein Research 36, 275-280). This problem can be largely mitigated through the use of the monoprotected phosphoserine derivative FmocSer[~~PO(OBzl)OHJ~~ [PO(OBzl)OH] (Wakamiya, T., Saruta, K., Yasuoka, J., and Kusumoto, S. (1994)[[.]] An Efficient Procedure For Solid-Phase Synthesis of Phosphopeptides ~~By~~ by the Fmoc Strategy[[.]], Chemistry Letters, 1099-1102). This derivative and the analogous threonine derivative have been applied to the synthesis of a range of peptides (White and Beythien, 1996). It has been noted with these derivatives too, that coupling of consecutive residues is sluggish. Stepwise incorporation of phosphoamino acid derivatives is not possible with Boc-based peptide synthesis, since the iterative Boc cleavage conditions, treatment with TFA, would degrade the phosphoryl derivatives.

Please amend the paragraph beginning on page 4, line 10 and extending to page 5, line 2 as follows:

Global phosphorylation involves the post-synthetic phosphorylation of unprotected hydroxyl residues on the solid support (Andrews, D. W. (1991). International Journal of Peptide and Protein Research 38,469.; Kitas et al., 1991; Otvos, L., Elekes, ~~I~~I., and Lee, V. M. Y. (1989)[[.]] Solid-Phase Synthesis of Phosphopeptides. International Journal of Peptide and Protein Research 34, 129-133.; Perich, J. W., and Johns, R. B. (1988)[[.]] Australian Journal of Chemistry 43,1623) or in solution (Perich, J. W., and Johns, R. B.(1988)[[.]]Di-Tert-Butyl N,N- Diethylphosphoramidite and Dibenzyl N,N- Diethylphospboramidite-Highly Reactive Reagents For the Phosphite-Triester Phosphorylation of Serine-Containing Peptides[[.]], Tetrahedron Letters 29, 2369- 2372). The post-synthetic phosphorylation strategy provides both the phosphorylated and unphosphorylated peptide in the same synthesis. In this approach, residues to be phosphorylated are most commonly incorporated without protection of the hydroxyl groups. This generally requires the use of less active acylating agents to avoid hydroxy acylation (Andrews, 1991; Debont, H. B. A., Vanboom, J. H., and Liskamp, R. M. J. (1990)[[.]] Automatic Synthesis of Phosphopeptides ~~By~~ by Phosphorylation ~~On~~ on the Solid-Phase[[.]], Tetrahedron Letters 31, 2497-2500; Kitas et al., 1991; Otvos et al., 1989; Perich et al., 1991). Side reactions have been reported (Yon, M. (1994)[[..]] In Innovations and Perspectives in Solid Phase Synthesis, 1993: Biological and Biomedical Applications[[.]], R. Epton, ed. (Birmingham: Mayflower Worldwide Ltd.), pp. 707), and the potential for undesired reactions increases with each coupling cycle. Thus, it is desirable to protect the reactive hydroxyl functionalities until phosphorylation. Complications in the coupling phase of the synthesis can be avoided by protecting the hydroxyl side chains as the trityl ethers. The trityl group can be removed without affecting the typical resin linkage used in Fmoc-based solid state peptide synthesis or other acid labile protecting groups; thus enabling a selective solid supported global phosphorylation step.

Please amend the paragraph on page 5, at lines 16-19 as follows:

The synthesis of sulfate-containing peptides is generally more difficult than that of the analogous phosphorylated peptides. The difficulties are at least in part due to the greater acid lability of the aryl sulfate ester (Fields, G. B., Tian, Z., and Barany, G. (1992)[[..]] In Synthetic Peptides. A User's Guide, G. A. Grant, ed. (New York: W. H. Freeman and Co.), pp. 77-183).

Please amend the paragraph on page 6, lines 12-28 as follows:

Kitagawa and coworkers reported the synthesis of CCK-12 using an orthogonal protection scheme for sulfation of a single tyrosine residue in partially protected peptide Futaki, S., Taike, T., Akita, T., and Kitagawa, K. (1992)[[.]] Synthese of 2 Tyrosine Sulfate Containing Peptides, Leucosulfakinin (LSK)-II and Cholecystokinin (CCK)-12, Using the ~~0~~O-Para-~~04~~-ethylsulphinyl)Benzyl Serine ~~For~~ for the Selective Sulfation of Tyrosine. Tetrahedron 48, 8899-8914) employing a safety catch-type protecting group to block alcoholic groups during peptide synthesis. The p-methylsulfinyl benzyl ether (Msib)ether group was used to block a serine side chain, and the corresponding carbamate (Msz) was used as ~~theN-terminal~~ the N-terminal α-amino protecting group. The p-methylsulfinyl benzyl group withstands treatment with TFA, but upon reduction to the thioether becomes acid labile. Standard Fmoc-based peptide synthesis using t-Bu protected tyrosine ~~were~~ was employed to assemble the protected dodecapeptide, which was then cleaved from the resin using TFA which also removed all other protecting groups except the p-methylsulfinyl ~~bezyl~~ benzyl groups. Free tyrosine was then sulfated with ~~DMF•SO$_3$~~ DMF·SO_3 complex and the sulfinyl groups were reduced with ethane dithiol. The Asp and Trp side chains were not reprotected before sulfation. The protected, sulfated peptide was purified by gel-filtration and final deprotection was accomplished using 90% aqueous TFA. A 6% yield of sulfated peptide after HPLC purification was reported.

Please amend the paragraph on page 16 at lines 25-31 as follows:

Figure 2 is a scheme for the synthesis of a protected tyrosine monomer having an azide-bearing protecting group exemplified for the synthesis of the compound of Figure 1A. In the scheme illustrated, conditions for step a are KO*t*-Bu, NaI, CH_3SCH_2Cl, DMF, specific yield for **3** is 82%; for step b, e.g. to make **4**, are: NCS, TMSCl, CH_2Cl_2; for step c to make **5** are NaN_3, DMF, H_2O, with specific yield over steps b and c to make **5** of 87%; for step d: TMSOTf, CH_2Cl_2, for step e: FmocOSu, Et_3N, THF (in synthesis of **1**, the yield over steps d and e was 84%) and for step f: LiOH-H_2O, ~~THF-H20~~ THF-H_2O, 0°C (with a specific yield for **1** of 88%).

Please amend the paragraph on page 17, line 1 as follows:

Figure ~~3is~~ 3 is an exemplary scheme for sulfated peptide synthesis.

Please rewrite the paragraph on page 18, lines 19-23 as follows:

The doctoral thesis of T. Young, (2001) University of Wisconsin (Madison) which is incorporated by reference herein, also contains a discussion of solid phase peptide synthesis (SPPS) particularly Boc-based and Fmoc-based methods and further contains a discussion of the 2-clorotrityl linker used in solid phase peptide synthesis. The thesis provides examples of protecting groups useful for side group protection in SPPS.

Please rewrite the paragraph bridging pages 19 and 20 as follows:

It may be the case that the reaction conditions for removal of a first and a second protecting group are mutually orthogonal, i.e., the reaction conditions selected for

removal of the first protecting group do not remove the second protecting group and the reaction conditions selected for removal of the second group do not remove the first group (e.g., where one group is acid labile and the other base labile). For example, each side-chain protecting group employed and any α-amino protecting group employed in peptide synthesis may be removable using mutually orthogonal reaction conditions. Similarly, it may be that conditions selected for removal of a selected protecting group do not cleave the peptide from a selected resin and that selected reaction conditions for cleavage of the peptide from a selected resin do not remove the selected protecting group. The term "mutually orthogonal" is used herein to refer to this ~~two way~~ two-way orthogonality of reaction conditions for removing different protecting groups and cleavage of the peptide from a support.

Please correct the paragraph at page 20, lines 4-14, as follows:

As used herein, the term "quasi-orthogonal" (including substantially quasi-orthogonal) includes situations in which a first bond (attaching a protecting group or linking a peptide to a solid support) can be cleaved under conditions that do not cleave a second bond (attaching another protecting group or linking a peptide to a solid support), but that any conditions sufficient for cleavage of the second bond would also cleave the first bond. For example, a first protecting group may be selectively removed, in relation to a second protecting group, under weakly acidic conditions, while the second protecting group may only be removed under more strongly acid conditions that also remove the first protecting group. The term orthogonal is intended to encompass both one-way and two-way orthogonality. "Mutually orthogonal" refers to ~~two way~~ two-way orthogonality of reaction conditions of two steps and ~~"quasi orthogonal"~~ "quasi-orthogonal" refers to one-way orthogonality of reaction conditions of two steps.

Please correct the paragraph on page 20, lines 16-24 as follows:

Reaction conditions refer to all reaction parameters that may be varied to change reactivity, including time, temperature, solvent, choice of reagent or reagents, concentrations of reactants and/or reagents, addition or removal of catalyst, change of catalyst, pH [[.]], ionic strength, pressure, etc. In principle, reaction conditions can be orthogonal because of a variation in any reaction parameter, but more typically are orthogonal because they employ different types of reagents (e.g., an acid vs. a base), different strengths of reagents (e.g., a weakly reducing agent vs. a strongly reducing agent) different pH or different concentrations of reagents (e.g., low concentration of acid vs. high concentration of acid.)

Please rewrite the paragraph on page 23, lines 13-16 as follows:

Methods for sulfation useful in combination with the selective deprotection methods provided herein are known in the art. Specific sulfation reagents that can be applied include, among others, any SO_3 equivalent including SO_3 (e.g., DMF·SO_3, ~~Pyridiine~~ pyridine·SO_3, Me_3N·SO_3), a tertiary ammonium salt of acetyl sulfuric acid (see: U.S. patent 4,444,682), and DCC/H_2SO_4.

Please rewrite the paragraph on page 23, lines 27-28 as follows:

In specific embodiments, the invention relates to selective sulfation of tyrosine ~~resideus~~ residues in peptides or proteins.

Please rewrite the paragraph on page 26, lines 4-16 as follows:

Initial screening of precedented methods for the selective activation of the O,S-acetal ~~3~~ **3** (T. Benneche, K. Undheim, Acta Chem. Scand. B 1983, 37, 93-96; P.J. Garegg, In Advances in Carbohydrate Chemistry and Biochemistry 1997, 52, 179-205) failed to yield satisfactory results. For example, treatment of compound **3** with NCS in dichloromethane afforded only 48% yield of the desired compound, **4**. Unsuccessful attempts to transform the O,S-acetal at high yield prompted a search for more reactive electrophilic activators. It was found that Lewis acid activation of N-chlorosuccinimide increased the consumption of starting material without compromising the stability of the product. Indeed, activation of the O,S-acetal with N-chlorosuccinimide in the presence of TMSC1 provides the chloride **4** in good yield (Figure 2). The labile intermediate **4** was purified by flash chromatography on deactivated silica gel, but not without some accompanying hydrolysis. A simple aqueous workup, however, provided **4** with little decomposition. The transformation of this material to the azide **5** proceeds in high yield (87% for 2 steps).

Please rewrite the paragraph bridging pages 27-28 as follows:

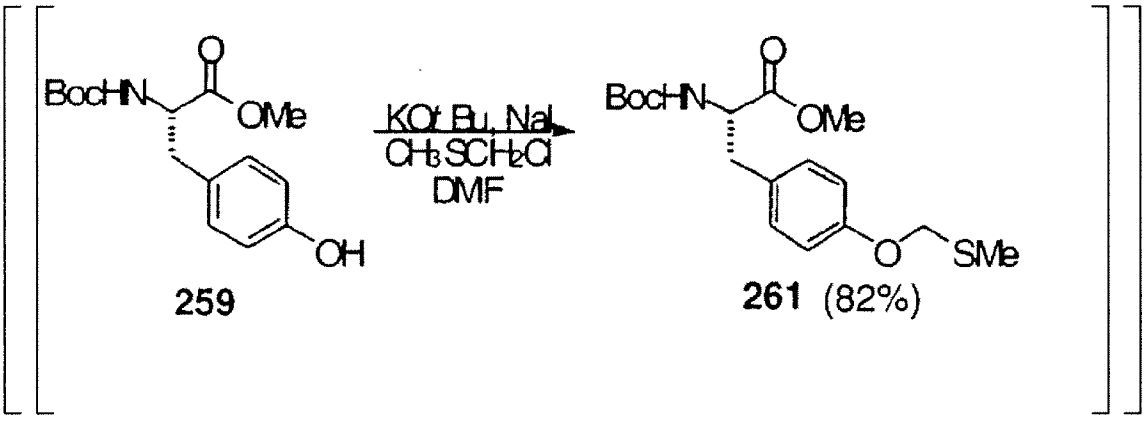
A peptide of sequence corresponding to residues 5-12 of mature PSGL-1 that contains all three putative sulfated tyrosine residues was synthesized. To demonstrate the flexibility of the inventive strategy, the fully sulfated octapeptide and a monosulfated ~~octapeptide7~~ octapeptide **7** (Figure 4) were synthesized. Synthesis of the precursor to the tri-sulfated sequence **6** via automated continuous flow Fmoc-based chemistry proceeded smoothly. Amide bond formation with the inventive monomer building block proceeds with efficiency similar to that observed with other amino acid monomers; no special reagents or conditions were required. After the post-synthetic operations of Azm cleavage, sulfation, resin cleavage, and benzyl group hydrogenolysis, the desired peptide **6** was isolated in 27% yield after HPLC purification. Importantly, no desulfated peptide was observed in either the MALDI-TOF mass spectrum (prior to HPLC) or

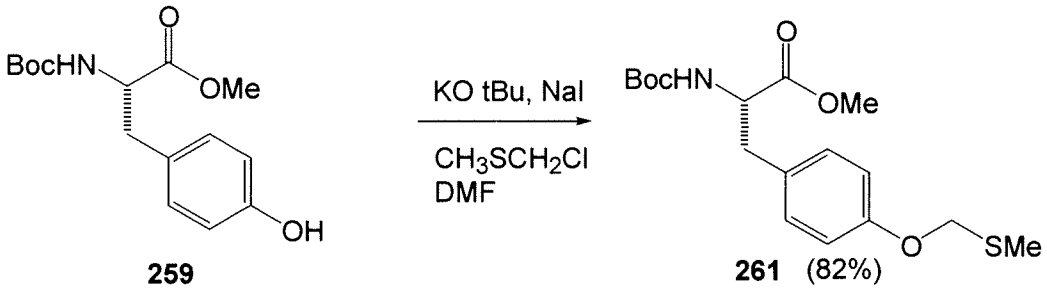
during HPLC purification. Synthesis of the monosulfated peptide **7** proceeded somewhat less efficiently, yielding several apparent deletion peptides in addition to the desired product. Still, after HPLC purification, the target peptide **7** was isolated in 5% overall yield based on resin loading. No desulfated peptide was detected.

Please rewrite the paragraph bridging pages 30 and 31 as follows:

B. Detailed Synthetic Procedures

BocTyr(MTM)OMe:



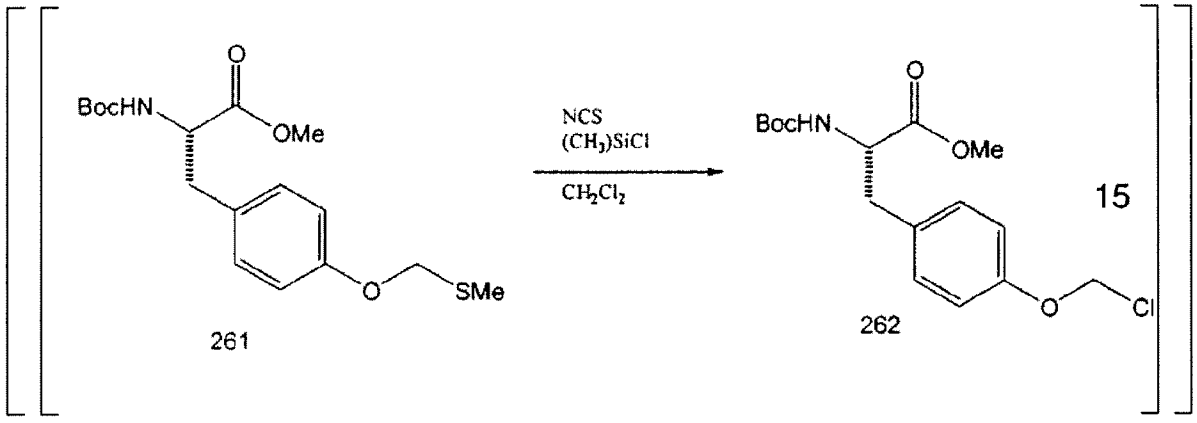


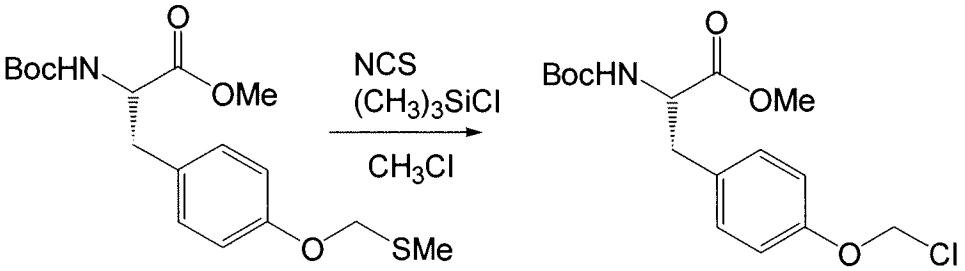
To a solution of BocTyrOMe (4.05 g, 13.7 mmol) and NaI (206 mg, 1.37 mmol) in DMF (30 mL) chilled via an external ice bath was added a THF (15 mL) solution of potassium *t*-butoxide (1.73 g, 15.1 mmol). To the resultant phenoxide (clear green solution) ~~was added~~ chloromethyl methyl sulfide (1.33 mL, 15.1 mmol) was added slowly. The reaction was allowed to warm gradually to room temperature. After 4.5 h,

the reaction was cloudy and TLC analysis (4:1 hexanes/EtOAc) indicated complete consumption of starting material. The reaction mixture was diluted with EtOAc (60 mL) and washed with H_2O (1 x 45 mL), aqueous citric acid solution (5%, 1 x 45 mL) and brine (1 x 45 mL). The aqueous washing were pooled and washed with EtOAc (2 x 60 mL). The combined organic extracts were pooled and dried over $MgSO_4$. The $MgSO_4$ was removed by filtration and volatiles removed *in vacuo*. The residue was purified by flash column chromatography (silica, gradient elution 4:1 hexanes/EtOAc to 2:1 hexanes/EtOAc) to afford a clear syrup on concentration ~~261~~ **261**(3.99 g, 81 %). $\mathbf{R_f}$ = .44 (4:1, hexanes/EtOAc); **IR** (Neat): 3368, 1744, 1714, 1510 cm^{-1}; ^{1}**H NMR** (300 MHz, $CDCl_3$): δ 7.02-6.81(AA'BB', J = 9.5, 4H), 5.06 (s, 2H), 4.99 (d, J = 5.4, 1H), 4.49 (q, J = 5.8, 1H), 3.66 (s, 3H), 2.18 (s, 3H), 1.36 (s, 9H); ^{13}**C NMR** (75 MHz, CDC1y): δ 172.2, 156.0, 130.3, 129.1, 116.0, 72.3, 54.4, 52.2, 37.5, 28.3, 14.4; **LRMS** (ESI): *m/z* 378 [M+Na^+ calc'd for $C_{17}H_{25}NO_5S$ 378.1]

Please rewrite the paragraph on page 31 lines 9-31 as follows:

BocTyr(CH_2)Cl:



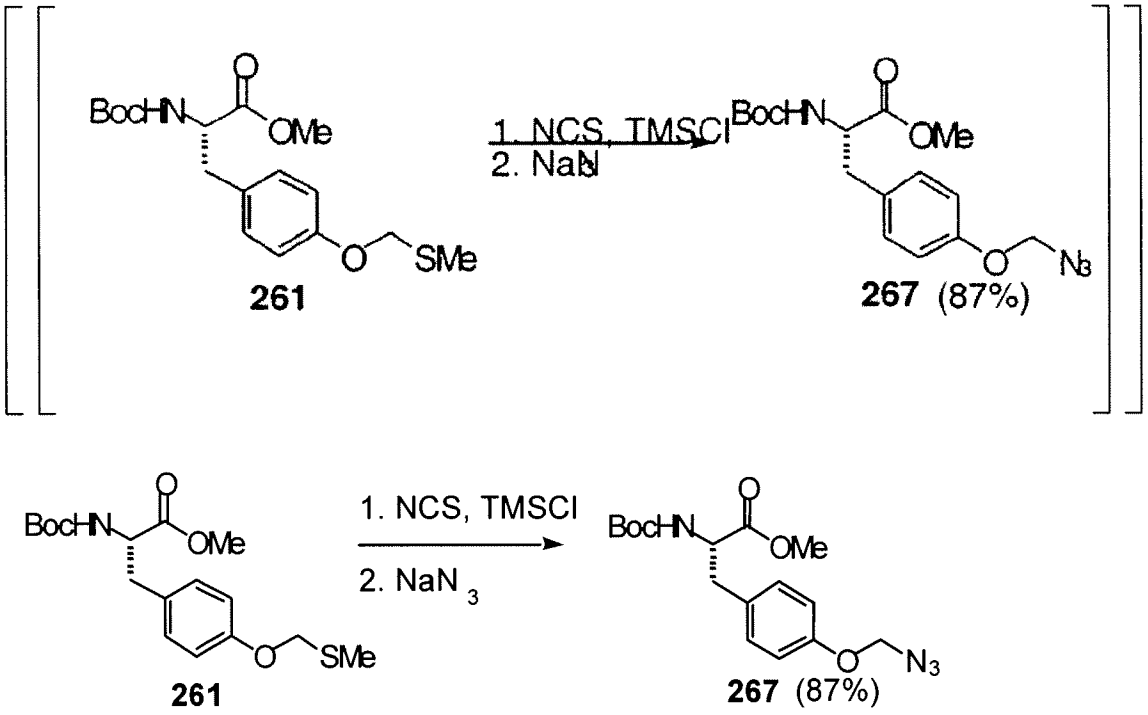


261 **262**

The *O,S*-acetal 261 (287 mg, 0.81 mmol) was dissolved in dichloromethane (3.0 mL), and solid NCS (119 mg, 0.89 mmol) was added. The reaction was allowed to stir for 2.5 h, then trimethylsilyl chloride (.11 mL, 0.89 mmol) was added. After an additional 2 hours, the crude reaction mixture was loaded directly on to a flash silica gel column. Elution with 5:1 hexanes/EtOAc and drying *in vacuo* provided the compound as a clear oil in pure form for characterization. The compound **262** was crystallized to yield white plates under high vacuum for 10 h (202 mg, 73%). The mass balance was recovered as BocTyrOMe after elution with EtOAc. $\mathbf{R_f}$ = .40 (4:1, hexanes/EtOAc); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.10-6.98 (AA'BB', J = 8.8 Hz, 4 H), 5.84 (s, 2H), 4.98 (d, J = 7.7 Hz, 1H), 4.53 (q, J = 7.8 Hz, 1H), 3.68 (s, 3H), 3.05- 2.95 (m, 2H), 1.38 (s, 9H);**^{13}C NMR** (75 MHz, $CDCl_3$): δ 172.1, 154.6, 130.4, 116.1, 54.3, 52.1, 37.4, 28.1; **LRMS** (MALDI, α-cyano-4-hydroxycinnamic acid matrix, positive ion mode): *m/z* 368.2, 366.1, 318.2 [calc'd M+Na^+ for $C_{16}H_{22}ClNO_5$ 366.11; M+Na^+ for $C_{15}H_{21}NO_5$ 318.13].

Please rewrite the paragraph bridging pages 31 and 32, as follows:

BocTyr(Azm)OMe:

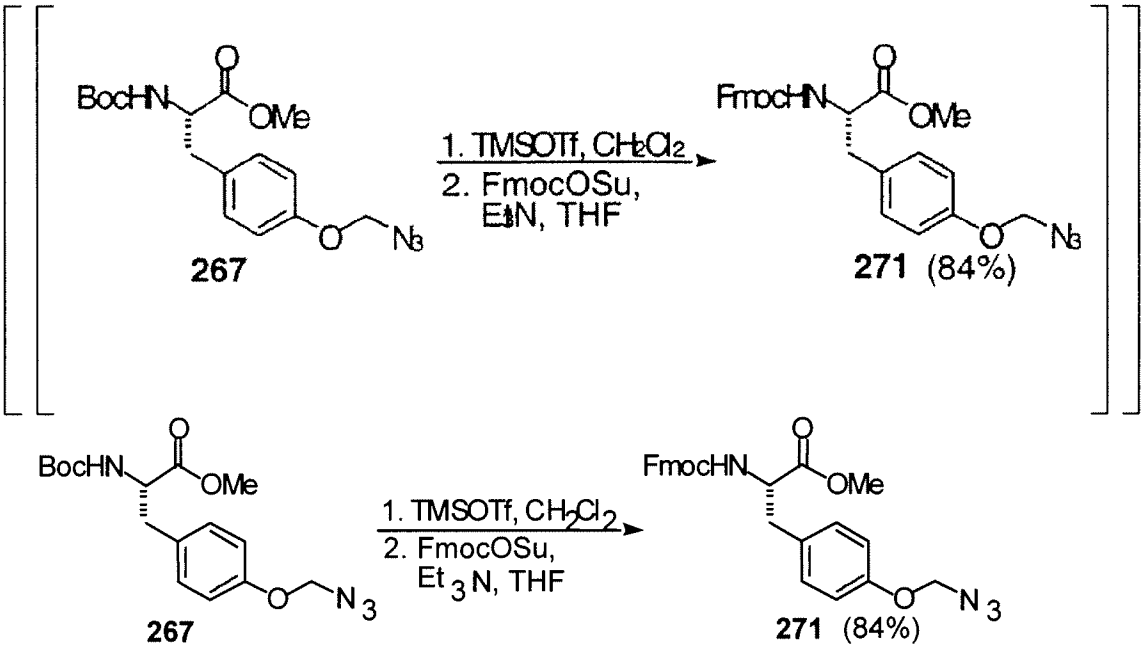


The *O,S*-acetal **261** (4.28 g) was dissolved in CH_2Cl_2 (35 mL) and solid N-chlorosuccinimide (1.76 g) was added. The reaction was allowed to stir at room temperature for 4 h. Trimethylsilyl chloride (1.68 mL) was then added slowly. After an additional 6 h, the reaction was diluted with $CHCl_3$ (30 mL) and saturated $NaHCO_3$ solution (60 mL) was added. The organic layer was separated and the aqueous fraction was extracted with $CHCl_3$ (2 x 60 mL). The combined organic extracts were concentrated via rotary evaporation and the residue dissolved in DMF(15 mL). Sodium azide (1.2 g, 18.5 mmol) was dissolved in H_2O (15 mL) and added to the solution of crude tyrosyl chloride. This reaction was allowed to stir for 5 h at room temperature. The reaction was then diluted with saturated $NaHCO_3$ solution (15 mL) and washed with EtOAc (3 x 30 mL). The combined organic extracts were dried ($MgSO_4$) concentrated *in vacuo*, and the residue subjected to flash column chromatography (silica, gradient elution 4:1 hexanes/EtOAc to 2:1 hexanes/EtOAc). After removal of volatiles, azidomethylene **267** was isolated as a clear oil (3.64 g, 87%). $\mathbf{R_f}$ = .41 (4:1

hexanes/EtOAc); **IR** (Neat): 2132, 2110 cm '; **^{1}H NMR** (300 MHz, $CDCl_3$): $\underline{\delta}$ 7.08-6.90 (AA'BB', J = 8.5 Hz, 4 H), 5.13 (s, 2H), 4.95 (d, J = 6.7 Hz, 1H), 4.55 (d, J =6.7 Hz 1H), 3.71 (s, 3H), 3.0 (m, 2H), 1.41 (s, 9H); **^{13}C NMR** (75 MHz, $CDCl_3$): $\underline{\delta}$ 172.1, 155.4, 130.3, 115.8, 79.6, 54.3, 51.9, 37.2, 28.0; **LRMS** (FAB): *m/z* 373.1 [M+Na^+ calc'd for $C_{16}H_{22}N_4O_5$ 373.2]

Please correct the paragraph bridging pages 32 and 33, as follows:

FmocTyr(Azm)OMe:

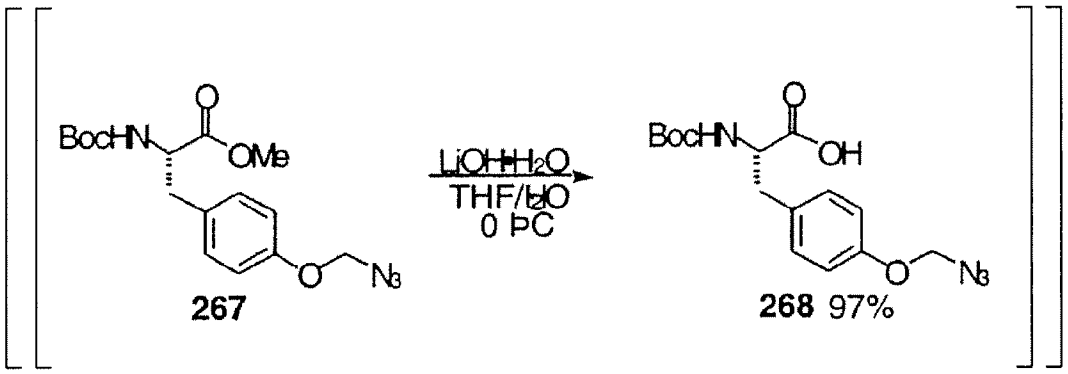


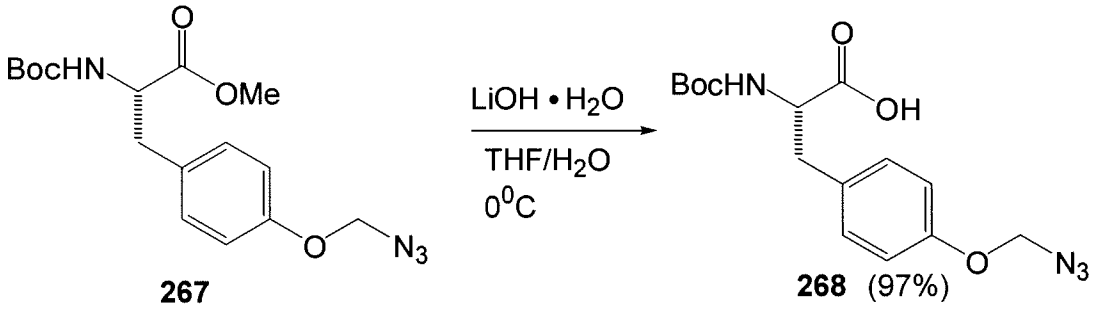
Boc protected compound **267** (769 mg, 2.19 mmol) was dissolved in CH_2Cl_2 and cooled with an external ice bath. TMSOTf (0.79 mL, 4.4 mmol) was added dropwise. TLC analysis (4:1 Hexanes/EtOAc) indicated complete consumption of starting material after less than 10 min. 5% aqueous Na_2CO_3 was added (15 mL), followed by EtOAc (15 mL). The organic layer was separated and the aqueous phase extracted with EtOAc (3 x 15 mL). Volatiles were removed *in vacuo* and the residue was taken up in THF (7 mL). Triethylamine (0.91 mL, 6.6 mmol) was added, followed by solid FmocOSu (814 mg, 2.4

mmol). The reaction was left to stir for 3.5 h during which time a white precipitate formed. The reaction was diluted with $CHCl_3$ (15 mL) and washed with H_2O, 5% citric acid solution, and brine (15 mL each). The combined aqueous washings were extracted with $CHCCl_3$ (4 x 15 mL). The pooled organic extracts were dried over $MgSO_4$ and filtered. The solvent was reduced to ca. 5 mL via rotary evaporation and loaded directly on a silica gel column. Flash chromatography (2:1 hexanes/EtOAc) yielded the compound **271** as a crystalline white solid (870 mg, 84%). Ry= .36 (2:1 hexanes/EtOAc); **^{1}H NMR** (300 MHz, $CDCl_3$): $\underline{\delta}$ 7.76-7.74 (d, J = 7.7 Hz, 2H, 7.57-7.53 (m, 2H), 7.41-7.36 (t, J = 7.1 Hz, 2H), 7.36-7.23 (t, J = 7.3 Hz, 2H), 7.02-6.88 (AA'BB', J = 7.4 Hz, 4H), 5.28 (d, J = 8.1 Hz, 1H), 5.09 (s, 2H), 4.63 (dd, J = 7.0, J = 10.6, 1H), 4.44 (dd, J = 7.0, J = 10.6, 1H), 4.16 (t, J = 7.0, 1H), 3.71 (s, 3H), 3.13-2.99 (m, 2H); **^{13}C NMR** (75 MHz, $CDCl_3$): $\underline{\delta}$ 171.7, 155.6, 143.7, 141.2, 130.4, 129.8, 127.6, 126.9, 126.8, 124.9, 119.9, 115.9, 79.6, 66.7, 54.7, 52.2, 47.0, 37.7; **LRMS** (ESI): m/z 495.1 [M+Na^+ calc'd for $C_{26}H_{24}N_4O_5$ 495.16]

Please correct the paragraph bridging pages 33 and 34 as follows:

BocTyr(Azm)OH:



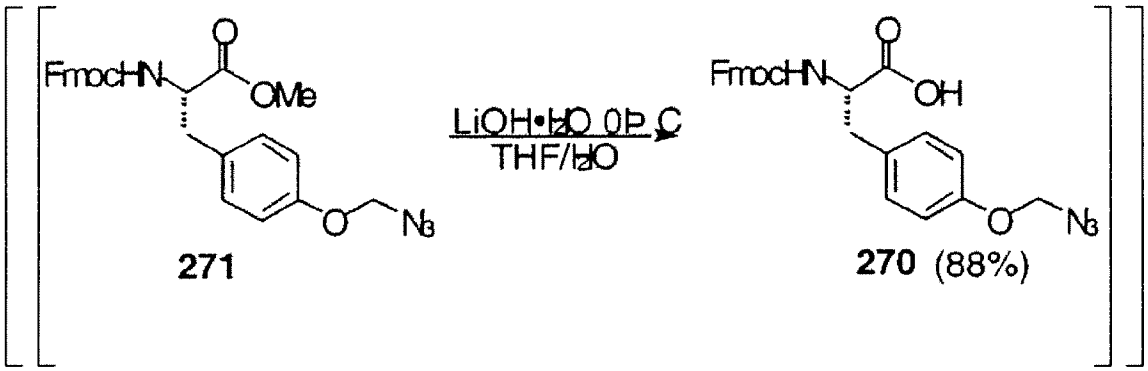


267 **268** (97%)

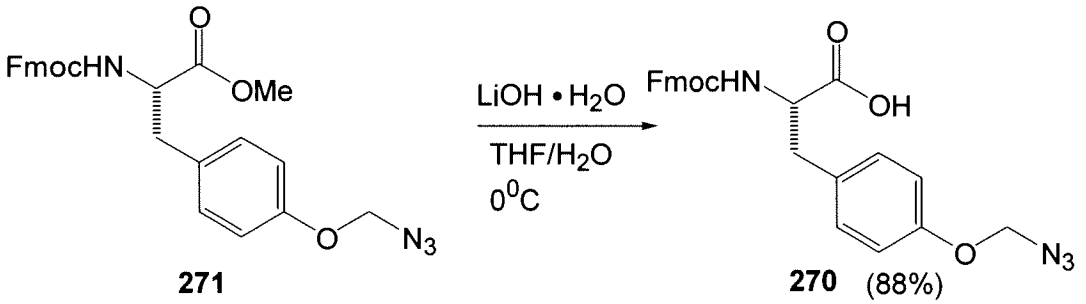
Methyl ester **267** (622 mg, 1.78 mmol) was dissolved in THF (5 mL) and cooled with an external ice bath. $LiOH \cdot H_2O$ (224 mg, 5.34 mmol) was dissolved in H_2O (5 mL) and chilled in the ice bath. The LiOH solution was then added to the methyl ester in one aliquot. After stirring at 0 °C for 2h TLC analysis (4:1, hexanes/EtOAc) indicated complete consumption of starting material. The reaction was diluted with 5% aqueous citric acid solution (20 mL), yielding an apparent pH of 3 (pH paper). The aqueous solution was extracted with EtOAc (4 x 20 mL). The combined organic extracts were pooled and dried over $MgSO_4$. Filtration and removal of volatiles *in vacuo* provided **268** as a white foam suitably pure (according to TLC, ^1H NMR) for analytical characterization (584 mg, 97%). **^1H NMR** (300 MHz, $CDCl_3$): δ 8.75 (br s, 1H), 7.12-6.90 (AA'BB', J = 6.9 Hz, 4H), 6.45 (m, minor rotamer NH) 4.96 (d, 7.7 Hz, major rotamer NH) 4.55 (m, major rotamer αH, .61/1H), 4.33 (m, minor rotamer αH, .38/1H), 2.95-2.80 (m, major rotamer, β2H), 1.40 (s, major rotamer, *t*-Bu), 1.29 (s, minor rotamer, *t*-Bu).

Please replace the paragraph bridging pages 34 and 35 as follows:

FmocTyr(Azm)OH:



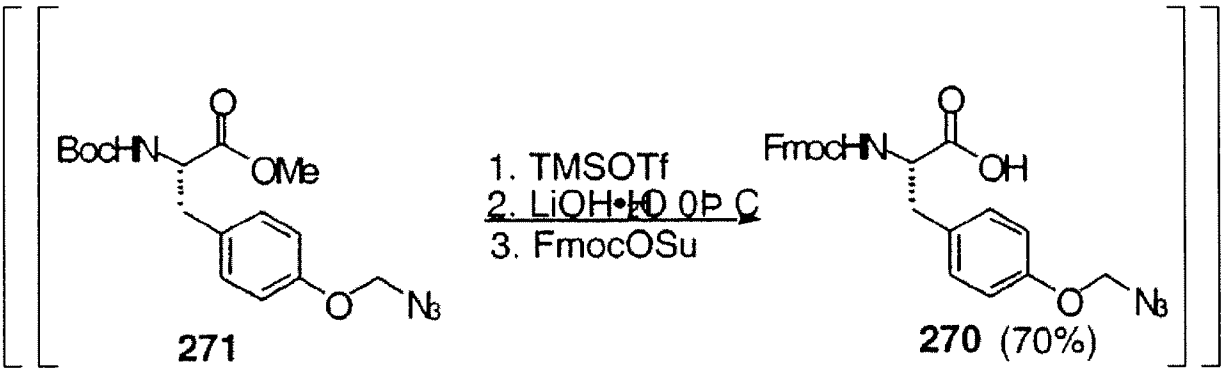
271 **270** (88%)

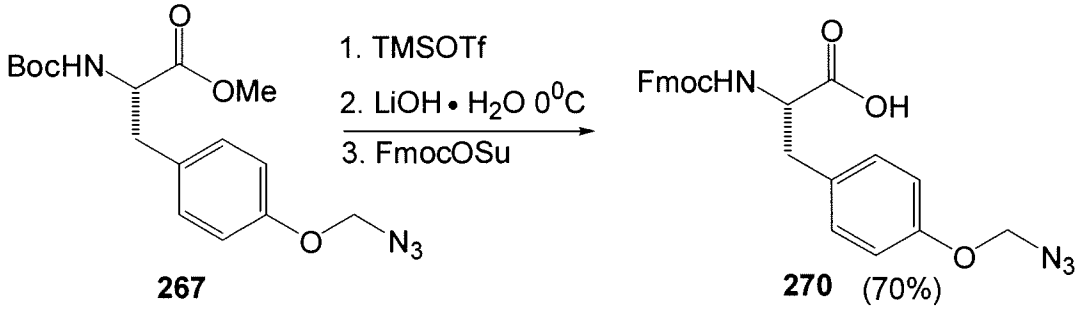


Methyl ester **271** (339 mg, 0.72 mmol) was dissolved in THF (7 mL) and cooled to 0 °C with an external ice bath. $LiOH{\cdot}H_2O$ (60 mg, 1.4 mmol) was dissolved in H_2O (7 mL) and added dropwise over 10 minutes to the chilled solution of methyl ester. After an additional 25 min the starting material was completely consumed as judged by analytical TLC (2:1 hexanes/EtOAc). The pH was then adjusted to ca. 3 by adding 0.3 M aqueous HCl. The cloudy aqueous solution was extracted with EtOAc (4 x 15 mL). The organic extracts were pooled, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash silica gel chromatography (10 % MeOH/$CHCl_3$) provides **270** as a white solid after drying *in vacuo* (292 mg, 89%). **^{1}H NMR** (300 MHz, $CDCl_3$): $\underline{\delta}$ 10.05 (br s, 1H), 7.78 (d, J = 7.7 Hz, 2H), 7.59-7.52 (m, 2H), 7.41 (t, J =7.4 Hz, 2H), 7.32 (t, J = 7.4 Hz, 2H), 7.09-6.90 (AA'BB', J = 6.9 Hz, 4H), 6.20 (m, minor rotamer NH), 5.43 (d, J = 8.1 Hz, major rotamer NH), 5.08 (s, 2H), 4.67 (q, J = 6.6, 1H), 4.52-4.46 (m, 1H), 4.40-4.34 (m, 1H), 4.23-4.17 (m, 1H), 3.23-3.05 (m, major rotamer $\underline{\beta}$H), 2.98-2.80 (m, minor rotamer $\underline{\beta}$H); **^{13}C NMR** (75 MHz, $CDCl_3$): $\underline{\delta}$ 155.4, 147.8, 143.3, 140.9, 130.2, 127.4, 126.7, 126, 124.6, 79.3, 66.7, 46.7, 36.5; **LRMS** (ESI): m/z 457.1, 235.1 [MH calc'd for $C_{25}H_{22}O_5N_4$ 457.15; MH calc'd for $C_{25}H_{22}O_5N_4$ – $C_{15}H_{11}O_2$ 235.1].

Please rewrite the paragraph on page 35 lines 9-26 as follows:

FmocTyr(Azm)OH, Method B:





Boc Tyr(Azm)OMe ~~271~~ 267 (766 mg, 2.19 mmol) was dissolved in CH_2Cl_2 (10 mL and cooled with an external ice bath. Trimethylsilyl triflate (0.79 mL, 4.4 mmol) was added dropwise over 3 minutes. After an additional 15 minutes, TLC analysis (5:1, hexanes/EtOAc) indicated complete consumption of starting material. Aqueous Na_2CO_3 solution (5%, 25 mL) was added, followed by 10 mL $CHCl_3$. The two phases were separated and the aqueous phase washed with EtOAc (4 x 25 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to give 2.1 g of an oil. This crude material was dissolved in THF (10 mL) and chilled via an external ice bath. A chilled solution of $LiOH \cdot H_2O$ (276 mg, 6.57 mmol) in H_2O (10 mL) was added. After 2 hours acetic acid (2.5 eq) was added and the ice bath was removed. Solid FmocOSu (740 mg, 2.19 mmol) was added, and this mixture was allowed to stir for 2 hours. The bulk of the THF was then removed via rotary evaporation and triethylamine (1 mL) was added. The crude aqueous mixture was filtered through a medium sintered glass frit and the solid washed with H_2O (3 x 15 mL). The combined aqueous mixture was acidified to an

apparent pH of 2 (pH paper) with 1 N HCl and washed with EtOAc (4 x 60 mL). The combined organic extracts were concentrated and the residue purified by flash column chromatography (silica, gradient elution 95:5 $CHCl_3$/MeOH to 80:20 $CHCl_3$/MeOH). Drying *in vacuo* yields ~~the270~~ **270** as an off-white, chalky solid (678 mg, 1.53 mmol, 68%). Analytical data (TLC, ^{1}HNMR, ^{13}CNMR, LRMS) same as above.

Please rewrite the paragraph on page 36 lines 12-19 as follows:

The stability of the carboxylate – chlorotrityl bond is enhanced by deblocking of the α-amino group. Thus, the Fmoc group is cleaved by rinsing of the resin with 10% piperidine/CH_2Cl_2 (2x), followed by 20% piperidine/DMF for 20 minutes. The resin is agitated via sparging with nitrogen gas during this reaction. At the conclusion of the Fmoc cleavage the resin is rinsed, DCM (3X), DMF (2X), iPrOH (2X), DMF (2X), iPrOH (2X), MeOH (2X), and Et_2O (2X). Solvent volume for all washes is 8 mL per gram of resin. The resin is then dried under high vacuum and stored at sub-zero temperatures. In general, superior loadings are achieved using this protocol relative to commercially available, pre-loaded resins.

Please rewrite the paragraph bridging pages 36 and 37 as follows:

Peptide Synthesis:

Synthesis using FmocTyr(Azm)OH for the introduction of sulfotyrosine residues was carried out on 25 μM scale using an automated synthesizer (Applied Biosystems Model 432A "Synergy"). Standard techniques were used (Fields, G. B., and Noble, R. L. (1990)[[.]] Solid-Phase Peptide-Synthesis Utilizing 9-Fluorenylmethoxycarbonyl Amino-Acids[[.]], International Journal of Peptide and Protein Research 35[[.]], 161-214; Merrifield, R. B. (1963)[[.]] Solid Phase peptide Synthesis I[[.]], The synthesis of a Tetrapeptide[[.]], Journal of the American Chemical Society 85, 7129-~~7 133~~ 7133.) The first amino acid was attached as above or the pre-loaded resins were purchased from

Advanced Chemtech (Louisville, Kentucky). Carboxylic and alcoholic side chains were protected with benzyl groups. Each synthesis cycle is initiated with the cleavage of the Fmoc group from the α-amino group, using 20% piperidine in DMF. Three equivalents (75 μmol) of the amino acid to be coupled is dissolved in DMF and added to the resin cartridge with HBTU (2-(1H-benzotriazol-l-yl)-1,1,3,3 tetramethyluronium hexafluorophosphate) and HOBt (N- hydroxybenzotriazole). The reaction cartridge is subjected to continuous flow conditions during each reaction. Following the coupling of the final amino acid, the peptide – resin cartridge is removed from the synthesizer. All subsequent manipulations of the peptide – resin are performed manually. Subsequent ~~reaction~~ reactions are agitated by the "double syringe" method. Briefly, a luer lock syringe is attached to each end of the peptide synthesis cartridge and the syringes are moved reciprocally and in tandem to agitate the reaction.

Please rewrite the paragraph on page 38 lines 22-31 as follows:

Ac YA: This dipeptide was synthesized manually using the double syringe method. Fmoc-L-Ala was attached to 2-Clt resin according to the standard procedure. Fmoc cleavage was followed by coupling of FmocTyr(Azm) (PyBop, DIPEA) according to the method of Castro ~~et. al.~~ et al. Fmoc cleavage and acetylation was followed by cleavage from the support. HPLC purification [Vydac C18, gradient elution: A = ~~.1%~~ 0.1%TFA/H_2O, B: CH_3CN/~~.1%~~ 0.1%TFA 0 – 20% B/30min t_2 = 26.76. Excision of the peaks and weighing on an analytical balance revealed relative peak size of 92.3:7.7 (84.6% ee). The synthesis of this dipeptide was repeated using Carpino's amide bond forming conditions (HATU, HOAt, collidine). All other steps were performed in the same way. This synthesis yielded material with a relative peak size of at least 95:5 (>90% ee).

Please rewrite the paragraph on page 39, lines 1-3, as follows:

^{1}H NMR (300 MHz, CD_3OD): δ 8.35 (d, J = 6.5 Hz, amide NH), 7.10-6.69 (AB q, J = 6.4 Hz, 4H), 4.58 (dd, J = 4.3, J = 5.0, 1H), 4.43-4.36 (m, 1H), 3.11-2.73 (m, 2H), 1.90 (s, 3H), 1.41 (d, J = 7.5, 3H).

Please rewrite the paragraph on page 39, lines 5-9, as follows:

Ac Y-(D)-A: HPLC purification [Vydac C18, gradient elution: A = ~~.1%~~ 0.1%TFA/H_2O, B: CH_3CN/~~.1%~~ 0.1% TFA 0 – 20% B/30min t_2 = 21.4 min. Excision and weighing of the peaks gave a ratio of 92.4:7.6 (84.8% ee). The synthesis of this dipeptide was repeated, as above, using Carpino's amide bond forming conditions (HATU, HOAt, collidine). This synthesis yielded material with a relative peak size of at least 95:5 (>90% ee).

Please rewrite the paragraph on page 39, lines 11-19, as follows:

Ac$Y_sEY_sLDY_sDF$: The peptide was synthesized on 0.025 mmol scale by standard coupling procedures. Commercially available pre-loaded resin (~~.5~~ 0.5 mmol/g) was used. The N-terminal Fmoc group was cleaved and the amino group acetylated. The azidomethylene group was cleaved in the usual way. Sulfation was performed as described above. Cleavage and lyophilization affords 28 mg of a white solid. HPLC purification [Alltech Econosil C18, one major peak: t_2 = 22.76 min, gradient system: CH_3CN/~~.1~~ 0.1 M aq. NH_4OAc 5% – 75% in 40 min, 8 mL/min] affords 8 mg (27% based on resin loading, minus resin for characterization) of a flocculent white solid. **IR** (KBr): 1244 br, str, 1050 br, str; **LRMS** (MALDI, α-cyano-4- hydroxycinnamic acid matrix, negative ion mode): m/z 1138.3 [calc'd M-3SO_4+NH_4^+ 1138.48].

Please rewrite the paragraph on page 39, lines 21-27, as follows:

AcYEYLDYDF: [[ ]]
A fraction of the phenol-deprotected material (10 mg resin) from the synthesis of $AcY_sEY_sLDY_sDF$ above was cleaved (yield 6 mg), dissolved in $MeOH/H_2O$ (2 mL) and subjected to hydrogenation over Pearlman's catalyst (10 mg) for 12h under an H_2 filled balloon. Filtration through pre-rinsed Celite ($MeOH/H_2O$, 1:1 eluant) afforded 3 mg crude material after lyophilization. **LRMS** (FAB -α-cyano-4-hydroxycinnamic acid matrix, positive ion mode): m/z 1215.4 [calc'd MH+ $2Na^+$ 1215.44].

Please rewrite the paragraph bridging pages 39-40 as follows:

AcYEY$_2$LDYDF: The solid phase synthesis was performed according to the general procedures described above. Cleavage from the resin gave 17 mg of crude peptide. This material was subjected to hydrogenation over Pearlman's catalyst (20 mg) for 12h under an H_2 filled balloon. Filtration through pre-rinsed Celite (H_2O eluant). This material was subjected to HPLC (Alltech Econosil C18) gave three major peaks, two of which appeared to be deletion peptides (by MALDI-MS, we were unable to assign a structure based on the mass spectra, however the peptides appeared to be sulfated, as judged by HPLC retention time). The longest retained peptide (t_r = 33.48 min, gradient system: CH_3CN/~~.1~~ 0.1 M aq. NHqOAc 5% – 75% in 40 min, 8 mL/min] pooling of this HPLC fraction and lyophilization afforded the desired peptide as a fluffy white solid (4.6 mg, 5.2 %) **LRMS** (MALDI, α-cyano-4-hydroxycinnamic acid matrix, negative ion mode): *m/z* 1170.4 [calc'd M-SO_4^-+~~NH_4~~ NH_4^+ 1169.42]; (MALDI, 2,4,6- trihydroxyacetophenone, negative ion mode): *m/z* 1191.6 [calc'd M – SO_3 + Na^+ for $C_{57}H_{68}N_8O_{22}S$ 1191.41] **IR** (KBr): 1256 br, str, 1049 br, str.

Please rewrite the paragraph on page 40, lines 12-15, as follows:

$Ac Y_{Bn} E_{Bn} YL D_{Bn} Y_{Bn} D_{Bn} F$: After azidomethylene deprotection of the above peptide-resin a small portion was cleaved (8 mg resin) to yield ca. 2.5 mg of intermediate crude peptide. **LRMS** (FAB, α-cyano-4-hydroxycinnamic acid matrix, positive ion mode): m/z 1642.6 [calc'd MH + Na^+ 1642.69] Other lower molecular weight peaks were observed, but were not assignable.

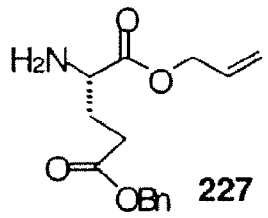
Please rewrite the paragraph on page 40, lines 7-22, as follows:

Cleavage of sulfated peptides from chlorotrityl resin:

The resin is dried under high vacuum for two hours before the cleavage reaction is attempted. Dichloromethane/trifluoroethanol/acetic acid cleavage solution (7:2:1 v:v:v, 10mL per gram of resin) is cooled to 0 °C and added to a flask containing dried resin in an ice bath. The mixture is stirred for 1.5 hours at 0 °C. During this time the ~~temperaturedoes~~ temperature does not exceed 5 °C. At the end of the reaction time the free peptide is filtered into a flask. The resin is then washed with the same volume of cleavage solution (at 0°C) used in the reaction. Both washes are combined and most of the solvent is evaporated on a rotary evaporator (water bath less than 10 °C). Ether (40 mL) is added to the residue, the peptide is pelletted on the centrifuge and the ether is decanted. This procedure is repeated for another ether wash (40 mL) and for an ethyl acetate/ ether wash (1.5:1 v:v, 25 mL total). The peptide pellet is redissolved in methanol, transferred to a flask, and evaporated to an oil (rotary evaporator water bath less than 10 °C). The oil is redissolved in methanol and evaporated to remove acetic acid. The oil is then lyophilized twice from MQ water to remove any traces of acetic acid. After removal of acetic acid, the crude peptide is stored at -25 °C until HPLC purification.

Please rewrite the paragraph bridging pages 41-42 as follows:

L-Glutamic acid γ-benzyl-α-allyl ester:



227

N-α-Fmoc-L-glutamic acid γ-benzyl ester-α-allyl ester was taken up in CH_2Cl_2/DMF (1:1, 20mL) and piperidine (7.5 mL) was added neat. After 25 minutes TLC analysis (2:1 Hex/EtOAc) indicated complete consumption starting material. The volume was reduced to ca. 2 mL in vacuo, dry Et_2O was added (30 mL) followed by concentrated HCl. A precipitate formed immediately and was filtered, washed with Et_2O and dried. The free base was obtained by dissolution in H_2O, neutralization with aqueous $NaHCO_3$ solution, extraction with EtOAc and drying *in vacuo*. This material was suitably pure for the next step. Analytically pure material was obtained by flash silica gel chromatography (of the free base 20 % MeOH-$CHCl_3$). **^{1}H NMR** (300 MHz, CDC1y): δ 7.35 (a s, 5H), 5.96-5. 87 (m, 1H), 5.36 (dd, J = 1.5 Hz, J = 7. 1 Hz, 1H), 5.28 (dd, J = 1.4 Hz, J = 10.0 Hz, 1H), 5.13 (s, 2H), 4.62 (a d, J = 5.7 Hz, 2H), 3.50 (dd, J = 5.1, J = 7.6 Hz, 1H), 2.53 (t, J = 7.5, 2H), 2.18-1.84 (m, 2H).

Please amend the paragraph at page 42, lines 12-24 as follows:

Fmoc $Y_sE_{Bn}OH$: Fmoc-L-glutamic acid γ-benzyl ester was attached to 2-Clt resin in the usual way. The resin loading was determined to be 0.75 mmol/g by quantitation of Fmoc cleavage. Resin (450 mg, 0.34 mmol) was subjected to the usual Fmoc cleavage conditions. FmocTyr(SO_3Na) was coupled using the general HATU-mediated coupling conditions described above [2 x (450 mg, ~~.7~~ 0.7mmol)] with an extended coupling time

(5h). Cleavage of the dipeptide from the resin using the general cleavage conditions afforded suitably pure dipeptide (yield 67%) as assayed by ^{1}HNMR and analytical HPLC [gradient 25:75 CH_3CN/~~.1~~ 0.1 M aqueous NH_4OAc – 75:25/30 min, at 3 mL/min; retention time: 24.7 min, Vydac C18]. **^{1}H NMR** (300 MHz, CD_3OD): δ 7.75 (d, J = 7.4, 1H), 7.56-7.21 (m, 17H), 5.03 (d, J = 2.6 Hz, 1H), 4.90 (s, 2H), 4.49 (aq, J = 4.8 Hz, 1H), 4.32 (td, J = 5.9, 2.6 Hz), 4.25-4.10 (m, 3H), 3.12-2.68 (m, 2H), 2.47-2.42 (m, 2H), 2.22-2.18 (m, 1H), 1.98-1.91 (m, 1H).

Please amend the paragraph bridging pages 42-43 as follows:

Fmoc$Y_SE_{Bn}Y_SLD_{Bn}Y_SD_{Bn}$F: The stepwise procedure was employed as described above for the first six residues. The hexapeptide was α-amino deprotected as usual. The dipeptide, FmocY_SE_{Bn}OH (2.3 equivalents), was then coupled to the support-bound hexapeptide using HATU and HOAt (5h). Cleavage of the octapeptide was accomplished in the usual way. HPLC [Econosil C18, gradient 25:75 CH_3CN/~~.1~~ 0.1 M aqueous NH_4OAc – 75:25/30 – 40min at 8 mL/min; retention time: 25.4 min] (11% yield based on resin loading). **^{1}HNMR** (300 MHz, CD_3OD) is consistent with the structure.

Please amend the paragraph on page 43, lines 3-8, as follows:

pEYyLDYDF: This peptide was generated in the attempted synthesis of Fmoc $Y_SEY_SLDY_SDF$ *via* the stepwise protocol described above. HPLC purification of the product and analytical characterization revealed the pyroglutamate-terminated structure. A satisfactory mass spectrum was not obtained for this compound. However, 2-dimensional ^{1}HNMR analysis (TOCSY, COSY) showed, unambiguously, this sequence. HPLC [Econosil C18, gradient 25:75 CH_3CN/ ~~.1~~ 0.1M aqueous NH_4OAc – 75:25/30 – 40min at 8 mL/min; retention time: 36.8 min].

Please rewrite the citation on page 56 at lines 25-26 as follows:

Kehoe, J. W., and Bertozzi, C. R. (2000). Tyrosine sulfation: a modulator of extracellular protein- protein interactions. Chemistry & Biology~~? R57-R61~~ 7, 57-61.

Please rewrite the citation on page 63 at lines 1-5 as follows:

Moore, K. L., Patel, K. D., Bruehl, R. E., Li, F. G., Johnson, D. A., Lichenstein, H. S., Curnmings, R. D., Bainton, D. F., and McEver, R. P. (1995). P-Selectin Glycoprotein [[ ]] Ligand-1 Mediates Rolling of Human Neutrophils on P-Selectin. Journal of Cell Biology 128, 661-671.

Please rewrite the citation on page 63 at lines 25-29 as follows:

Norgard, K. E., Moore, K. L., Diaz, S., Stults, N. L., Ushiyama, S., McEver, R. P., Cummings, R. D., and Varki, A. (1993). Characterization of a Specific Ligand For P- [[ ]] Selectin On Myeloid Cells – a Minor Glycoprotein With Sialylated O-Linked Oligosaccharides. Journal of Biological Chemistry 268, 12764-12774.

Please rewrite the citation on page 66, lines 23-28 as follows:

Ramachandran, V., Nollert, M. U., Qiu, H. Y., Liu, W. J., Cuinmings, R. D., Zhu, C.,-and McEver, R. P. (1999). Tyrosine replacement in P-selectin glycoprotein ligand-1 affects distinct kinetic and mechanical properties of bonds with P- and L-selectin. Proceedings

of the National Academy of Sciences of the United States of America 96, [[

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13771- ~~l 3776~~ 13776.

Please rewrite the citation on page 71, lines 19-21 as follows:

White, P., and Beythien, J. (1996).[[.]] In Innovations ~~8c~~ & Perspectives in Solid Phase Synthesis & Combinatorial Libraries, 4th International Symposium, R. Epton, ed. (Birmingham: Mayflower Scientific Ltd.), pp. 557.